Production of α-fetoprotein by hepatoma cells and some ampulla cells

—Studies by peroxidase antibody technique—

Toshio SHIKATA

Department of Pathology, Faculty of Medicine, University of Tokyo, Tokyo, Japan.

Localization of α-fetoprotein (AFP) in the hepatoma cells as well as hepatocytes was investigated by the peroxidase antibody technique under a light and electronmicroscope, in the course of hepatocarcinogenesis in rat induced by 3′methyl-4-dimethylaminoazobenzene (3′-Me-DAB).

The livers of rats fed with 3′-Me-DAB for 5 to 7 weeks and for 25 weeks were used as materials. The 5–7 week group showed its first appearance of AFP in the serum and the 25 week group showed the second

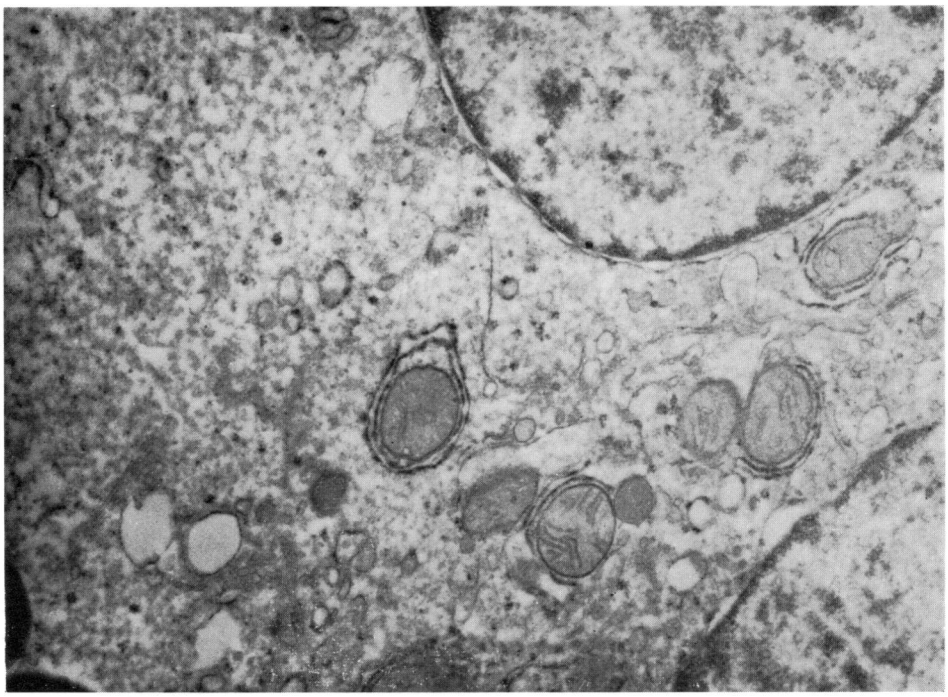

Fig. 1. Electron micrograph of AFP production in hepatoma cells using peroxidase reaction. Rough endoplasmic reticulum encompassing mitochondria showing positive reaction.

appearance of AFP in the serum. The liver specimens of the former case were observed under light microscope following the application of the peroxidase antibody method, and the latter case was observed under a light and electron microscope.

The specimen of the liver was fixed with 2% glutaraldehyde for 1 hr., washed and reacted with peroxidase-labeled anti-rat AFP serum for 12 hrs. at 4°C. Then the peroxidase reaction was applied. Observation under light microscope was done in this stage. For electronmicroscopical study, the specimen was refixed by osmic acid and embedded in Epon. The observation of ultrathin sections without staining was performed under a JEM 7 type electronmicroscope. Anti-rat AFP serum was supplied by Dr. Nishi (Hokkaido University).

AFP was demonstrated by this method around ribosomes of rough endoplasmic reticulum in the hepatoma cells. For the rough endoplasmic reticulum, the reaction was observed as an uneven, thick line along the membrane (Figs. 1–3). Unstained round granules which coincided with ribosomes were found in the thick black line. AFP was often found in the rough endoplasmic reticulum encompassing mitochondria.

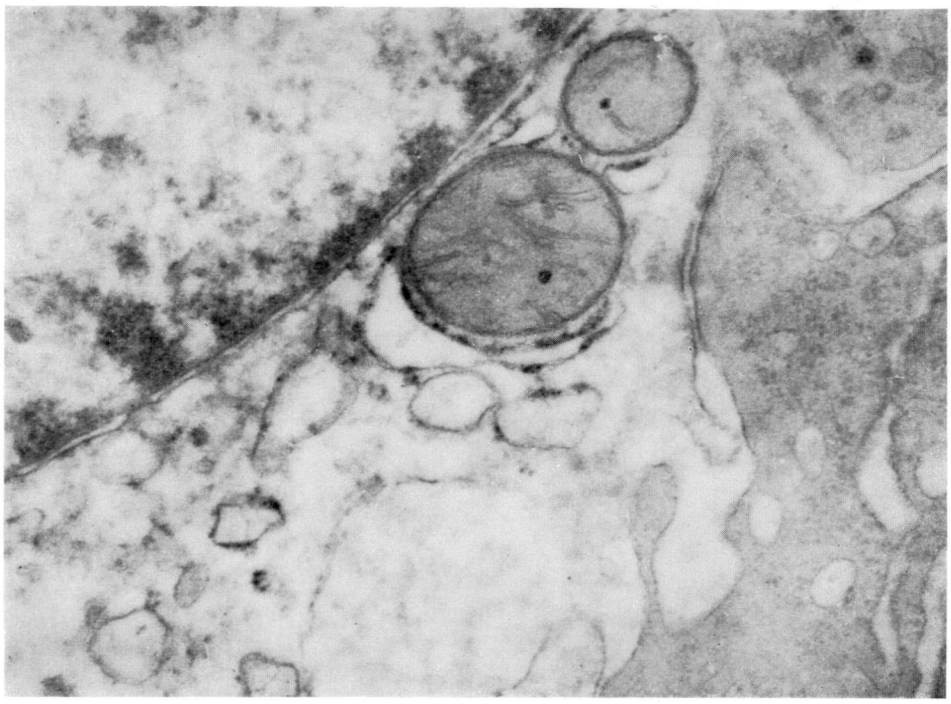

Fig. 2.

The secretory process of AFP appears to be excreted through the smooth endoplasmic reticulum to the cell surface, because in some areas, smooth endoplasmic reticulum and parts of cell membrane were stained. However, the Golgi apparatus did not seem to be related to the excretory process of AFP.

Initial reaction of AFP in the early stage of 3′-Me-DAB hepatocarcinogenesis was probably responsible for the proliferation of ampullar cells. This was demonstrated in the light-microscopic observation of the sections, which were stained by peroxidase labelled anti-rat AFP serum. Proliferated cholangiolar cells (so-called oval cells) did not show any evidence of AFP production.

On the other hand, studies on the relationship between serum AFP and histological and cytological patterns of malignant hepatoma in human beings disclosed two clear findings. One is that well-differentiated and highly anaplastic hepatoma did not produce AFP, or even if they do, the

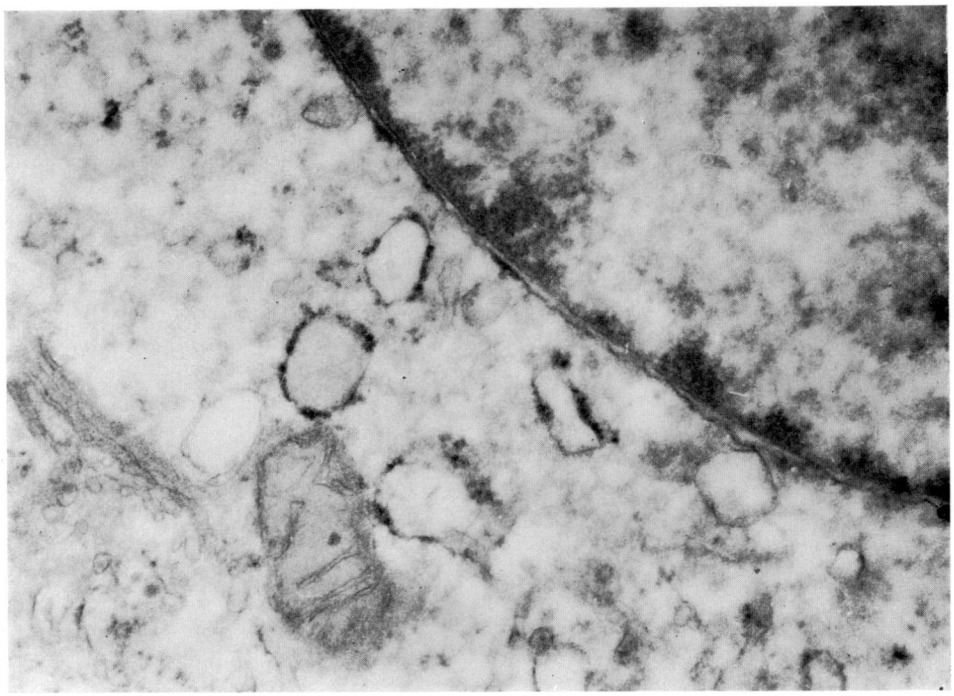

Fig. 3.

Fig. 2, 3. High magnification of hepatoma cells. AFP is demonstrated around ribosomes attached to rough endoplasmic reticulum. Ribosomes are seen as white spots in black lines. Golgi apparatus (lower left in Fig. 3) does not show any positive reaction.

serum level of AFP is very low. Second, except for well-differentiated and poorly differentiated groups, AFP titers of the serum have a positive correlation with dedifferentiation of malignant hepatoma.

REFERENCES

1. SHIKATA, T. and SAKAKIBARA, K.: Relationship between αf protein and histological patterns of hepatoma and localization of αf protein in the hepatoma cells using peroxidase antibody technique. *GANN Monograph on Cancer Research*, **14**, 269 (1973).
2. SHIKATA, T. and SAKAKIBARA, T.: α-Fetoprotein synthesis in the hepatoma cells. —Immunoelectron microscopic study— *Kanzo* (Acta Hepat. Jap.), **13**, 288 (1972). (in Japanese)
3. SHIKATA, T. and SAKAKIBARA, K.: α-Fetoprotein synthesis in 3′-Me-DAB hepatocarcinogenesis. *Proc. of the Jap. Cancer Ass. the 31st Annual Meeting*, 83 (1972).